

Product Sheet

H_APJ Reporter HEK-293 Cell Line

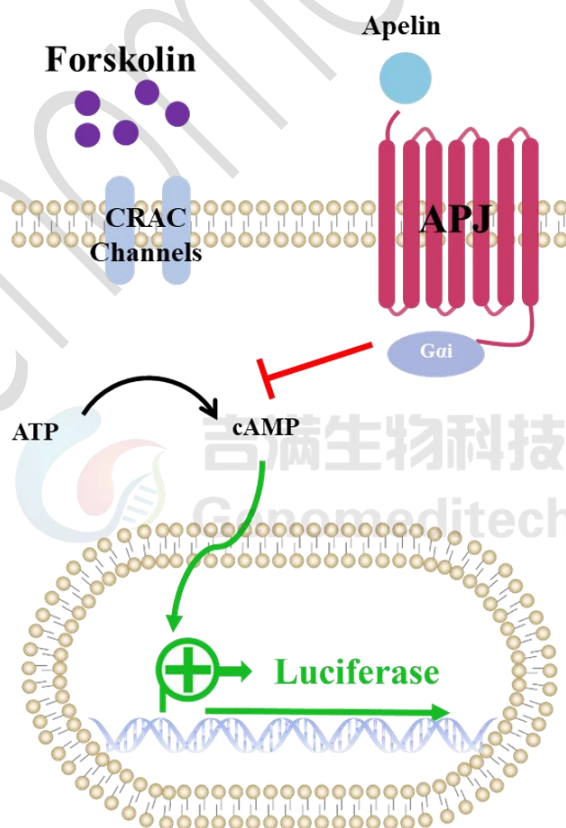
Catalog number: GM-C29525

Version 3.3.1.250425

The APJ receptor is a G protein-coupled receptor (GPCR) expressed on the cell membrane. It can bind to various ligands, but its most important endogenous ligand is Apelin. Apelin is a series of peptides encoded by the Apelin gene, which can bind to and activate the APJ receptor. There are multiple forms of Apelin peptides, including Apelin-36, Apelin-17, and Apelin-13.

When Apelin binds to APJ, the receptor activates G proteins (mainly Gai/o), leading to signaling pathways like reduced cAMP production, and activation of PLC/PKC and calcium signaling. The APJ-Apelin system is important for cardiovascular function, metabolism, and embryonic development.

H_APJ Reporter HEK-293 Cell Line is a clonal stable cell line constructed using lentiviral technology, constitutive expression of the APJ gene, along with signal-dependent expression of a luciferase reporter gene. When Apelin-13 binds to the APJ receptor, it activates downstream inhibitory signaling, thereby suppressing the Forskolin-induced increase in cAMP and resulting in decreased luciferase expression. The luciferase activity measurement indicates the activation level of the signaling pathway and can thus be used to evaluate the in vitro effects of related drugs.



Specifications

Quantity	5E6 Cells per vial, 1 mL
Product Format	1 vial of frozen cells
Shipping	Shipped on dry ice
Storage Conditions	Liquid nitrogen immediately upon receipt

Recovery Medium	DMEM+10% FBS+1% P.S
Growth medium	DMEM+10% FBS+1% P.S+4 µg/mL Blasticidin+0.75 µg/mL Puromycin
Note	None
Freezing Medium	90% FBS+10% DMSO
Growth properties	Adherent
Growth Conditions	37°C, 5% CO ₂

Mycoplasma Testing	The cell line has been screened to confirm the absence of Mycoplasma species.
Safety considerations	Biosafety Level 2
Note	It is recommended to expand the cell culture and store a minimum of 10 vials at an early passage for potential future use.

Materials

Reagent	Manufacturer/Catalogue No.
DMEM	Gibco/C11995500BT
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/ GM-040404
Puromycin	Genomeditech/ GM-040401
Forskolin	sigma/F6886
Apelin-13 TFA	MCE/HY-P1944A
Anti-APJ mIgG2a Antibody	//In house

Figures

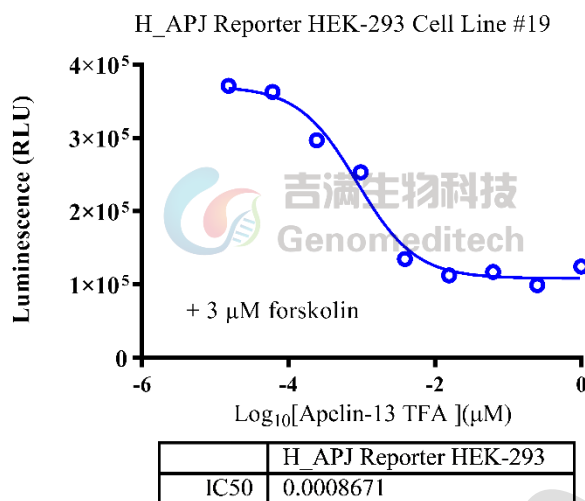


Figure 1 | Response to Apelin-13 TFA. Serial dilutions of the Apelin-13 TFA (MCE/HY-P1944A) was incubated with 1.5E4 cells/well of the H_APJ Reporter HEK-293 Cell Line (Cat. GM-C29525) in a 96-well plate for 1 hour in assay buffer (DMEM+1% FBS+1% P.S). Subsequently, the Forskolin (sigma/F6886) at a concentration of 3 μ M was added, and the coculture proceeded for an additional 16 hours. Firefly luciferase activity was then measured using the GMPOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated maximum blocking folds of approximately [3.7]. Data are shown by drug molar concentration.

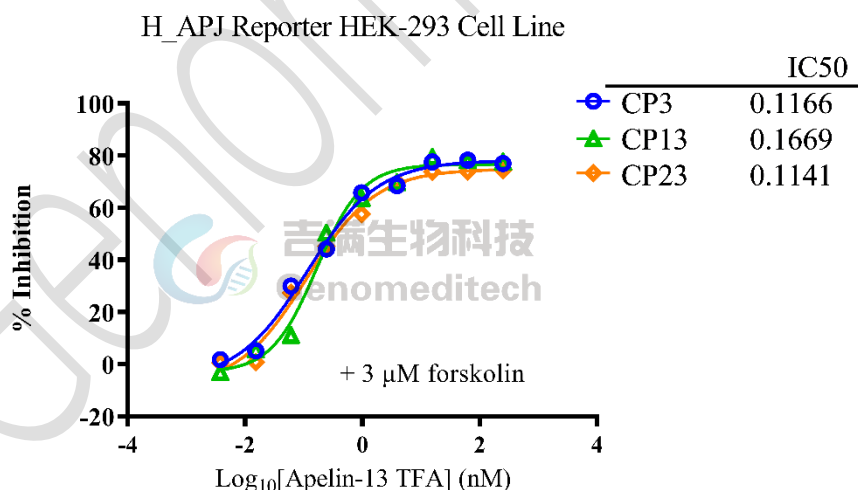


Figure 2 | The passage stability of response to Apelin-13 TFA. After serial dilution of Apelin-13 TFA (MCE/HY-P1944A), it was incubated for 1 hour in assay buffer (DMEM + 1% FBS + 1% P.S) with passage 3, 13, and 23 of the H_APJ Reporter HEK-293 cells (Cat. GM-C29525) at 1.5E4 cells per well in a 96-well plate. Subsequently, Forskolin (Sigma/F6886) was added to a final concentration of 3 μ M, and the coculture continued for an additional 16 hours. Firefly luciferase activity was then measured using the GMPOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). Data are shown by drug molar concentration.

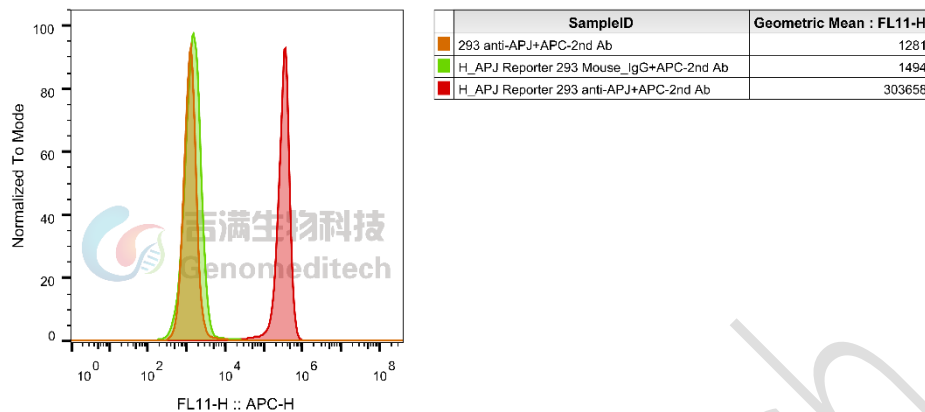


Figure 3 | H_APJ Reporter HEK-293 Cell Line (Cat. GM-C29525) was determined by flow cytometry using Anti-APJ mIgG2a Antibody (In house).

Cell Recovery

Recovery Medium: DMEM+10% FBS+1% P.S

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

- Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 - 3 minutes).
- Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
- Transfer the vial contents to a centrifuge tube containing 5.0 mL complete culture medium and spin at approximately 176 x g for 5 minutes. Discard supernatant.
- Resuspend cell pellet with the recommended recovery medium. And dispense into appropriate culture dishes.
- Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

Cell Freezing

Freezing Medium: 90% FBS+10% DMSO

- Centrifuge at 176 x g for 3 minutes to collect cells.
- Resuspend the cells in pre-cooled freezing medium and adjust the cell density to 5E6 cells/mL.
- Aliquot 1 mL into each vial.
- Place the vial in a controlled-rate freezing container and store at -80°C for at least 1 day, then transfer to liquid nitrogen as soon as possible.

Cell passage

Growth medium: DMEM+10% FBS+1% P.S+4 µg/mL Blasticidin+0.75 µg/mL Puromycin

For the first 1 to 2 passages post-resuscitation, use the recovery medium. Once the cells have stabilized, switch to a growth medium.

- Subculturing is necessary when the cell density reaches 80%. It is recommended to perform subculturing at a ratio of 1:3 to 1:4 every 2-3 days. Ensure that the density does not exceed 80%, as overcrowding can lead to reduced viability due to compression.
- Remove and discard culture medium.
- Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.
- Add 1.0 mL of 0.25% (w/v) Trypsin-EDTA solution to dish and observe cells under an inverted microscope until cell layer is dispersed (usually within 30 to 60 seconds at 37°C).
- Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
- Add 2.0 mL of growth medium to mix well and aspirate cells by gently pipetting.
- After centrifugation, resuspend the pellet and add appropriate aliquots of the cell suspension to new culture vessels.
- Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 - 1:4 is recommended

Medium Renewal: Every 2 to 3 days

Notes

- Upon initial thawing, a higher number of dead cells is observed, which is a normal phenomenon. Significant improvement is seen after adaptation. Once the cells reach a stable state, the number of dead cells decreases after subculturing and the cell growth rate becomes stable.
- Ensure that the cell density does not exceed 80%, as overcrowding may lead to reduced viability due to compression.

Related Products

NPR1	
H_NPR1 Reporter Cell Line	Cynomolgus_NPR1 CHO-K1 Cell Line
Flag-Mouse_NPR1 CHO-K1 Cell Line	H_NPR1 CHO-K1 Cell Line
H_NPR1 HEK-293 Cell Line	Mouse_NPR1 CHO-K1 Cell Line
Rat_NPR1 CHO-K1 Cell Line	
Anti-NPR1 hIgG1 Antibody(XX-16)	Anti-NPR1 hIgG1 Reference Antibody (XX-16)
Anti-NPR1 hIgG4 Antibody(REGN-5381)	Anti-NPR1 hIgG4 Reference Antibody (REGN-5381)

Limited Use License Agreement

Genomeditech (Shanghai) Co., Ltd grants to the Licensee all intellectual property rights, exclusive, non-transferable, and non-sublicensable rights of the Licensed Materials; Genomeditech (Shanghai) Co., Ltd will retain ownership of the Licensed Materials, cell line history packages, progeny, and the Licensed Materials including modified materials.

Between Genomeditech (Shanghai) Co., Ltd, and Licensee, Licensee is not permitted to modify cell lines in any way. The Licensee shall not share, distribute, sell, sublicense, or otherwise provide the Licensed Materials, or progenitors to third parties such as laboratories, departments, research institutions, hospitals, universities, or biotechnology companies for use other than for the purpose of outsourcing the Licensee's research.

Please refer to the Genomeditech Cell Line License Agreement for details.

Genomeditech